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Genetic Mapping of Ossification of the Posterior Longitudinal Ligament of the Spine

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Summary

Ossification of the posterior longitudinal ligament of the spine (OPLL) is recognized as a common disorder among Japanese and throughout Asia. Estimates of its prevalence are in the range of 1.9%–4.3%. Although its etiology is thought to involve a multiplicity of factors, epidemiological and family studies strongly implicate genetic susceptibility in the pathogenesis of OPLL. In this study we report an identification of a predisposing locus for OPLL, on chromosome 6p, close to the HLA complex. The evidence for this localization is provided by a genetic-linkage study of 91 affected sib pairs from 53 Japanese families. In this sib-pair study, D6S276, a marker lying close to the HLA complex, gives evidence for strongly significant linkage (P = .000006) to the OPLL locus. A candidate gene in the region, that for collagen 11A2, was analyzed for the presence of molecular variants in affected probands. Of 19 distinct variants identified, 4 showed strong statistical associations with OPLL (highest P = .0004). These observations of linkage and association, taken together, show that a genetic locus for OPLL lies close to the HLA region, on chromosome 6p.

Introduction

Ossification of the posterior longitudinal ligament (OPLL) in the spine is characterized by heterotopic bone formation in spinal ligaments, through endochondral mechanisms. It constitutes the leading cause of spinal-canal stenosis and myelopathy among Japanese, with a reported prevalence of 1.9%–4.3% (Matsunaga and Sakou 1997). This condition has also been recognized in outpatient clinics in the United States and Europe, where the prevalence is 0.01%–1.7% (Matsunaga and Sakou 1997). A related ossification disorder, diffuse idiopathic skeletal hyperostosis (DISH), or Forestier disease, has been described and characterized in Caucasian populations, by Resnick (1976). In DISH, bone proliferation occurs in spinal and extraspinal locations; however, in 50% of these cases, ossification of the posterior lateral ligaments was also found (Resnick et al. 1978). Recently, a DISH prevalence estimate of 25% in males and 15% in females, at age >50 years, has been found in a Caucasian population of consecutive patients, by means of posterior/anterior and lateral chest radiographs (Weinfeld et al. 1997). The extent of overlap of OPLL with DISH is unknown, but several investigators support the concept that OPLL represents a clinical subset of DISH (Trojan et al. 1992).

The average age at onset of OPLL is ~50 years in both men and women, although the male:female ratio is 2:1. Diagnosis of OPLL is confirmed by the finding of ossification of the spinal ligaments in transverse x-ray views of the cervical/thoracic/lumber vertebral column. Typical symptoms of this disease include sensory and motor dysfunction of the upper and lower extremities, bladder dysfunction, and abnormal reflexes. These myelopathies are likely to progress insidiously and chronically and are not relieved by conservative treatments. If myelopathy has progressed severely, then surgical intervention such as decompressive laminectomy or decompressive anterior fusion is recommended.

In spite of numerous investigations undertaken to clarify the various mechanisms involved in the ossification of the ligament, the primary determinants of OPLL have not been elucidated. Several lines of evidence—including pedigree studies, twin studies (Uehara et al. 1994), and an increased (28.9%) risk in siblings of affected individuals (Terayama 1989)—suggest the existence of genetic factors or loci responsible for the etiology of OPLL.

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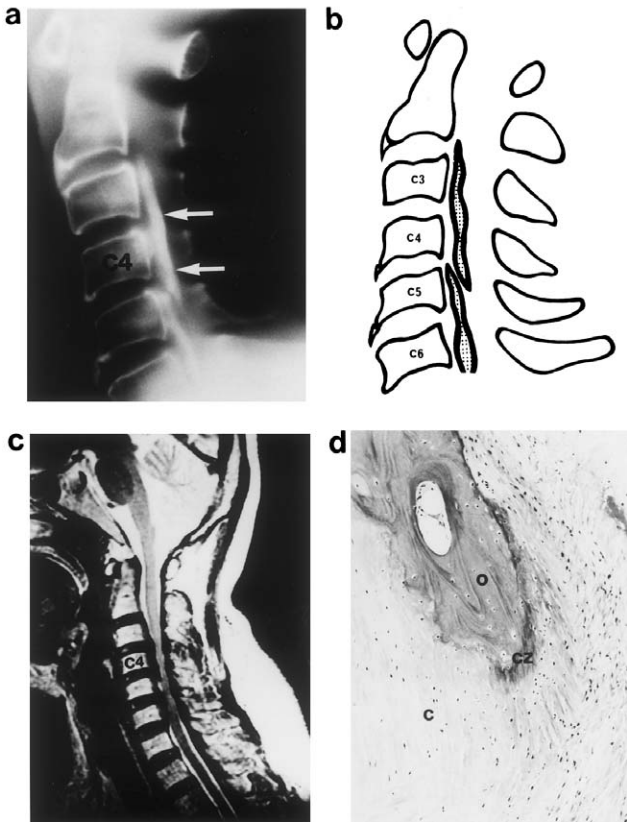

Figure 1 Radiological and histologic findings for OPLL. *a,* X-ray tomogram of the cervical spine from a typical OPLL subject. The heterotopic ossification of the posterior longitudinal ligament is observed between C3 and C6 (*arrows*). The patient showed myelopathy in upper and lower limbs, which was due to the compression of the spinal cord. *b,* Schematic drawing of x-ray radiogram findings. *c,* MRI, which clearly shows compression of the spinal cord (*arrowhead*). *d,* Typical histologic examination from an autopsy case with OPLL. The sagittal section of C3–C6 was stained with hematoxylin-eosin (magnification ×28). o = ossified tissue; cz = calcified zone; and c = cartilage tissue.

Relative risk to the sibling is estimated as 10. Segregation anaylses in families have supported both autosomal dominant and autosomal recessive patterns of inheritance (Terayama 1989; Uehara et al. 1994). In 9 of 39 sibships investigated by Terayama (1989), one parent was found to be affected. However, the mode of inheritance is obscured by a lack of large Japanese families, the late onset of the disease, a sex difference, and environmental effects. An important first step in understanding the role of genetic factors in OPLL is the report, by Sakou et al. (1991), of an allelic association ($P < .01$) of the HLA markers DR2 and Bw62 with OPLL. In addition, the siblings in that report, who had HLA haplotypes identical to those in the probands, showed a high incidence of OPLL.

On the basis of this reported HLA association, we used genetic-linkage methods to test the hypothesis that a gene predisposed to the OPLL phenotype resides on chromosome 6p. Seven highly informative dinucleotide markers at the HLA complex were selected for the genetic study. Since the mode of inheritance for OPLL is uncertain, we initially chose to use the nonparametric affected-sib-pair method, an analysis method independent of transmission model. Affected sib pairs were ascertained by five orthopedic centers in Japan. A total of 91 sib pairs from 53 sibships were analyzed.

A candidate gene for OPLL lies in the region under study. *COL11A2,* the gene for the collagen $\alpha 2$ (XI) chain, lies between the markers D6S291 and D6S273, in the same interval that contains the HLA-DR2 locus. We performed an extensive SSCP analysis on *COL11A2,* to screen the molecular variants contributing to the disease. Nineteen distinct variants thus far have been identified in *COL11A2.* Statistically positive associations between OPLL and several molecular variants were detected. For the purpose of predicting the disease-susceptibility allele in the gene, haplotype analysis based on maximum likelihood was performed. Estimated frequencies of haplotypes with four variants that exhibit positive associations were compared, between OPLL patients and control subjects. The haplotype should exhibit a variable degree of association with the causal mutation(s) and, thus, with OPLL. In fact, we detected strong associations with two specific haplotypes in OPLL subjects.

When taken together, these results—including genetic linkage, allelic association, and haplotype analysis—demonstrate that a genetic locus for OPLL lies close to the HLA region. Possible involvement of *COL11A2* in OPLL needs to be investigated further.

Subjects and Methods

Disease Criteria and Selection of Subjects

The diagnosis of OPLL was based both on the findings of symptoms by physical examination and on radiological findings (fig. 1). An individual was classified as affected if ossification was observed in any vertebrae of the cervical or thoracic portion of the spine. In general, the region of ossification occupied from 10% to >50% of the anterior part of the spinal canal. The ossification type was classified according to the criteria defined by the Investigation Committee on the Ossification of the Spinal Ligaments, Japanese Ministry of Public Health and Welfare. The appearance of OPLL observed in radiographs is classified into four types: (1) segmental, (2) continuous, (3) mixed, and (4) localized, circumscribed, or bridged. All four types of OPLL were identified in this study. The predominant type was segmental. All subjects were diagnosed by T.S, H.K., E.T, S.H., and

T.N. The study included 124 siblings, 46 females and 78 males, from 53 Japanese families. Subjects were recruited from five university hospital clinics, in Kagoshima, Wakayama, Asahikawa, Okinawa, and Hirosaki. All studies were conducted in accordance with human-subject standards set by the respective universities, and informed consent was obtained from the subjects prior to enrollment. Twenty-nine pairs, 12 trios, and 1 quartet were collected in Kagoshima; 4 pairs, 2 trios, and 1 quartet were collected in Hirosaki; 2 pairs were collected in Asahikawa; 1 pair was collected in Wakayama; and 1 pair was collected in Okinawa. The study samples comprise 91 pairs (46 females and 78 males). The affected sibships are composed of 37 pairs, 14 trios, and 2 quartets of OPLL siblings.

To determine accurately the allele frequencies in the general Japanese population, DNA samples were obtained from 212 healthy control (non-OPLL) individuals (136 females and 76 males) with normal spinal x-rays. The average age in this control group was 69.0 years (range 40–95 years). One hundred eighty-three controls (125 females and 58 males) were collected in Kagoshima, and 29 controls (11 females and 18 males) were collected in Hirosaki. To supplement the number of OPLL patients (affected sib pairs) in the association study with *COL11A2* gene polymorphisms, additional DNA samples were obtained from 137 patients (33 females and 104 males). The average age in this group was 60.6 years (range 41–81 years). One hundred twenty-one of these cases (29 females and 92 males) were collected in Kagoshima, and 16 (4 females and 12 males) were collected in Hirosaki.

Genotyping of Microsatellite Markers

DNA was extracted either from whole blood or from lymphoblastoid cell lines, by standard phenol-chloroform protocols. PCR reactions were performed in a 25-μl final volume containing 200 ng of genomic DNA, 200 μM each dNTP, 0.25 mM spermidine (Sigma Chemicals), 0.25 pmol of end-labeled primer, 10 pmol each of unlabeled forward and reverse primers, 1.25 μl of dimethyl sulfoxide, 0.1 U of *Taq* DNA polymerase, and 1 × PCR buffer (Boehringer Mannheim). Amplified products were loaded onto 7% polyacrylamide/8 M urea sequencing gels and were run for 4–5 h at 80 W. Samples from the same sibship were loaded into the gels in adjacent lanes. Gels were placed immediately on Hyperfilm MP (Amersham) and were exposed at −70°C for 16 h. On the basis of the films, genotypes were scored by two independent observers. A size standard derived from an M13 dideoxy sequencing ladder was included every 20 lanes, for accurate determination of allelic sizes. Alleles were scored according to their sizes (in base pairs). The number of observed alleles per marker system was 6–12. All markers had heterozygosities >70%, with the exception of D6S285, which had an observed heterozygosity of 41.3%.

SSCP Analysis of COL11A2

A total of 20 OPLL probands and 10 non-OPLL control subjects were initially screened for molecular variants. SSCP screening was performed on PCR-amplified segments spanning all 66 exons of the collagen gene, 2.6 kb of intron 1, and 0.6 kb of intron 6, as well as 1.3 kb of genomic sequence upstream of the transcriptional start site. A total of 165 primer sets were made to completely cover these genomic regions. These primer sets were designed from the human gene sequence, obtained from the EMBL-NEW5 database, accession number U32169 (primer sequences used in this study are available, on request, from the authors). PCR-SSCP analysis was performed as described elsewhere (Orita et al. 1989), with modifications. Samples were loaded onto a mutation detection–enhancement gel, according to the manufacturer's protocol (FMC). Gels were run in the presence or absence of glycerol, at room temperature. In order to screen GC-rich promoter segments, a mixture of dGTP (100 μM) and 7-deaza-2′-deoxyguanosine (100 μM) was added to the PCR reaction, according to the protocol of McConlogue et al. (1988). Variants detected by SSCP analysis were subjected to direct sequencing by a *Taq* DyeDeoxy Terminator protocol on an ABI 373A sequencing instrument (Applied Biosystems), as described elsewhere (Hata et al. 1990). When a variant was confirmed by DNA sequencing, the frequency of SSCP variants was compared between OPLL patients and controls. A total of 137 cases and 183 controls were typed for each SSCP polymorphism. The differences in the genotype frequency, between cases and controls, were analyzed by the χ^2 test statistic.

Linkage Analysis and Statistics

Identity-by-descent sib-pair analysis was performed by the SIBPAIR program from the computer package ANALYZE, version 2.1, by J. D. Terwilliger, obtainable at the Columbia Website. Allelic frequencies evaluated in the 138 Japanese non-OPLL controls, as described above (see the Genotyping of Microsatellite Markers section), were used in all linkage calculations. The haplotype frequencies were estimated by the maximum-likelihood method, by means of a simplified version of the computer program GENEF (Jeunemaitre et al. 1997).

Results

Clinical Features of OPLL

OPLL is usually diagnosed by radiographic methods, and the ossification is recognized along the posterior

margin of the vertebral bodies (figs. 1*a* and *b*). Most of the patients have some clinical symptoms, such as cervical pain or discomfort in conjunction with numbness of the upper limbs. With growth of the ossified mass, the spinal canal is compressed, resulting in severe myelopathy. Magnetic-resonance imaging (MRI) clearly demonstrates the compression of the spinal cord (fig. 1*c*). The typical results of histologic examination of an autopsy case with OPLL is shown in figure 1*d*. The sagittal section of C3–C6 shows the huge tumorlike ossified mass of the posterior longitudinal ligaments, growing longitudinally along the posterior aspect of the vertebral bodies. In front of the ossification foci, endochondral ossification of the calcified zone is observed. In this zone, different thicknesses bordering on the ossified tissue are seen. Numerous chondrocytes also accumulate in the remote area.

Linkage Analysis

We tested for linkage of OPLL to multiple markers in the chromosome 6p region, using the SIBPAIR program in ANALYZE. A total of 91 affected sib pairs from five different orthopedic centers in Japan were analyzed. Radiological findings were obtained on all patients. Because this analysis is sensitive to marker-allele frequency, we determined reference frequencies in a Japanese sample of healthy controls ($n = 212$) who had undergone x-ray examination and who were free of any ossification in their spinal ligaments. In all, seven dinucleotide-repeat markers flanking the HLA locus were tested for linkage to the disease phenotype. The order assumed for these markers is based on genetic and physical maps of this region (Martin et al. 1995; Feder et al. 1996; also see Whitehead Institute for Biomedical Research/MIT Center for Genome Research). From proximal to distal, the order is D6S426–(11 cM)–D6S291–(4 cM [interval includes HLA-DR locus])–D6S273–(1 cM)–D6S265–(<1 cM)–D6S276–(4 cM)–D6S299–(8 cM)–D6S285. Among these markers, significant genetic linkage was detected in the (identical by descent) sib-pair analysis ($P = 6 \times 10^{-6}$), with the marker D6S276, which is located distally to the HLA locus. The equivalent LOD score for this marker is 4.18, and the excess sharing of alleles is 14.8%.

Table 1 gives the results of the sib-pair analysis, for all seven markers. Haplotype analysis of all families shows that, at three marker loci—including D6S276, the marker with the most significant indication of linkage—three individuals (a female in kindred 18, a female in kindred 46, and a male in kindred 56) do not share the disease haplotype with their siblings. These three families are clear evidence that either more than one genetic locus exists for this condition or that nongenetic causes are also involved. If these families are excluded, then alleles are shared between all affected sib pairs ($n = 86$) in the 50 remaining families, for the adjacent markers D6S276 and D6S265. In an attempt to formally demonstrate locus heterogeneity, we analyzed the entire set of 53 families, for admixture among families, using the HOMOG test (Terwilliger and Ott 1994). For each of the seven marker loci tested, no significant evidence for a second locus was obtained; the most suggestive evidence for heterogeneity was obtained with marker D6S265 ($\chi^2 = 1$, df = 2.045, $P = .076$). Interestingly, four individuals within the families were excluded from the study because they did not meet the strict disease criteria of OPLL. Three individuals from separate families were diagnosed with ossification of the anterior ligaments of the spine, and one individual was diagnosed with ossification of other spinal ligaments (balsomy). When the haplotypes of all four individuals were examined, it was observed that all possessed the disease haplotype seen in their affected siblings.

Table 1

Sib-Pair Linkage Analysis (Identical by Descent) of the HLA Region

Locus	*P*
D6S285	.046
D6S299	.15
D6S276	.0000059
D6S265	.086
D6S273	.042
D6S291	.077
D6S426	.080

Mutation Search in the COL11A2 *Gene*

As a candidate gene, *COL11A2* was extensively screened for molecular variants in OPLL subjects. We performed a systematic SSCP analysis to screen for genetic mutations in all 66 exons and 1,300 bp of the 5′ noncoding region of the gene (Vuristo et al. 1995). In addition, regions known to be involved in splice variants—intron 6, intron 1, and a region associated with tissue-specific expression in the mouse (Lui et al. 1996; Tsumaki et al. 1996)—were also completely screened for mutations. Nineteen different molecular variants have been identified throughout the gene (table 2). Five of the variants (D593G, E824K, L879P, P1316T, and R1600Q) lead to amino acid changes in the coding regions. Among these variants, E824K and L879P were fairly frequent both in OPLL subjects and in controls, with no statistical differences between the groups (table 2). No mutation was detected in the key glycine residues of the triple-helix domain (exons 14–63) that could be the cause of a more severe phenotype, such as that observed in osteogenesis imperfecta (Francomano 1995). All 19 variants were subsequently detected in our control

Table 2

Polymorphisms in *COL11A2,* and Their Association with OPLL

Location (Position[a])	Nucleotide Substitution	Amino Acid Change	Allele Frequency[b]		χ^2 (df = 1)	*P*
			OPLL	Non-OPLL		
Promoter (−182)	A→C		.24 (*n* = 128)	.33 (*n* = 173)	5.10	.0240
Exon 1 (+24)	G→A	UTR	.06 (*n* = 93)	.06 (*n* = 70)	.02	.8825
Intron 1 (+1910)	G→A		.06 (*n* = 123)	.09 (*n* = 148)	1.39	.2390
Intron 6 (+353)	C→G		ND	ND		
Intron 6 (+608)	C→T		ND	ND		
Intron 6 (−4)	T→A		.14 (*n* = 129)	.26 (*n* = 152)	12.70	.0004
Intron 9 (+10)	A→G		ND	ND		
Exon 20 (+21)	A→G	D→G (593)	ND	ND		
Intron 20 (+32)	C→G		ND	ND		
Intron 22 (+70)	G→T		ND	ND		
Exon 27 (+21)	T→A	G→G (712)	.16 (*n* = 124)	.20 (*n* = 68)	1.05	.3050
Exon 32 (+36)	T→C	S→S (822)	ND	ND		
Exon 32 (+42)	G→A	E→K (824)	.17 (*n* = 106)	.15 (*n* = 89)	.41	.5230
Intron 35 (+3)	G→A		ND	ND		
Exon 36 (+42)	C→T	L→P (879)	.15 (*n* = 105)	.14 (*n* = 87)	.07	.7870
Exon 43 (+24)	A→G	P→P (1058)	.29 (*n* = 105)	.39 (*n* = 149)	5.37	.0210
Exon 46 (+18)	C→T	P→P (1128)	.15 (*n* = 130)	.22 (*n* = 172)	4.53	.0333
Exon 54 (+40)	C→A	P→T (1316)	ND	ND		
Exon 64 (+49)	G→A	R→Q (1600)	.03 (*n* = 116)	.02 (*n* = 66)	.40	.5288

[a] Indicates the nucleotide at the start of the exon or intron, with the exception of intron 6, in which the number refers to its position from the start of exon 7.

[b] ND = not determined (because of low frequency).

population; no obvious disease-causing mutation was identified. Allelic frequencies were compared between cases and controls; the results of this association study with *COL11A2* polymorphisms are presented in table 2. Statistically significant associations were detected with four molecular variants—an A→C substitution at position −182 in the promoter region ($\chi^2 = 1$, df = 5.10, $P = .024$), a T→A substitution at position 636 in intron 6 (4 bases upstream from the start of exon 7) ($\chi^2 = 1$, df = 12.70, $P = .0004$), an A→G substitution in exon 43 ($\chi^2 = 1$, df = 5.37, $P = .021$), and a C→T substitution in exon 46 ($\chi^2 = 1$, df = 4.53, $P = .033$).

Testing Haplotypes in OPLL Patients and Controls

Haplotype estimation was performed by maximum likelihood. When four polymorphisms (position −182, intron 6, exon 43, and exon 46) were examined jointly, maximum-likelihood analysis revealed marked linkage disequilibrium, with four predominant haplotypes. Estimated haplotype frequencies for the four predominant haplotypes (H1–H4) were compared between OPLL patients and controls (table 3). Surprisingly, for two of the four common haplotypes, significant differences between cases and controls were noted; one of these haplotypes (H1), consisting of all common alleles at each site, occurred at a higher frequency in cases than in controls ($\chi^2 = 1$, df = 7.38, $P = .0066$), and the other haplotype (H4), consisting of the rarer allelic counterparts, was significantly less common in cases than in controls ($\chi^2 = 1$, df = 16.86, $P < .0001$).

Discussion

Although our understanding of the pathogenesis of OPLL and related ossification processes has greatly increased in recent years, particularly with regard to the role of bone morphogenetic proteins, activins, and/or transforming–growth-factor interactions in ossification (Kawaguchi et al. 1992; Hayashi et al. 1997; Yonemori et al. 1997), the molecular cause of this common disorder remains unknown. Identification of the genetic susceptibility of the disease is the first step toward an understanding of the molecular pathogenesis of OPLL. It could provide a new biological insight into ectopic bone formation in the ligament.

We performed the genetic-linkage study in order to detect the locus of the disease. In the case of OPLL, a mode of inheritance has not been clearly established, and large-pedigree ascertainment is not practical, because of the late onset of the disease. A total of 91 affected sib pairs were selected from various orthopedic hospitals in Japan. HLA haplotype analysis with modest numbers of families suggested that the disease-susceptibility locus lies in the HLA region (Sakou et al. 1991). Affected-sib-pair linkage analysis of a nonparametric method was tested with seven markers at the HLA region. We detected, among the markers tested, both a strong linkage

with D6S276 and weaker linkages with other neighboring markers (table 1).

We considered the possibility that *COL11A2* could be a candidate gene for OPLL, because of its potential involvement in the ossification process and its chromosomal location, which is proximal to HLA class II, between the markers D6S276 and D6S291. The fact that *COL11A2* does not occur precisely at the peak of linkage could be due to differences in information content of the marker that contribute differently to the sib-pair linkage study. It is known that relatively small changes in the frequencies of rare alleles can dramatically alter linkage results. Therefore, our sib-pair linkage analysis might not necessarily pinpoint the precise genetic locus. In Alzheimer disease, apolipoprotein E4 is well recognized as a risk factor. Despite the fact that apolipoprotein-E association was highly and consistently significant, linkage analysis at the *ApoE/ApoC2* locus gave weak evidence for linkage (Pericak-Vance et al. 1991; Liu et al. 1996). The potential role of collagen in the pathogenesis of OPLL is supported by several lines of evidence. Typical findings during histopathological examination of the ossified ligament from OPLL subjects are proliferation of active chondrocytes at the area of the ossification front and thickening of the ligaments (Yamazaki and Goto 1992). This heterotopic bone formation is dominated by endochondral ossification. It is well recognized that collagen plays a key role in the ossification process and that the collagens α1(XI) and α2(XI) are the minor components of the extracellular matrix of chondrocytes (Line et al. 1993). Furthermore, the tiptoe-walking Yoshimura (twy) mouse, which has been considered as an animal model for OPLL, is characterized by hyperostosis of the ligaments, along with elevated expression of collagen α1(XI) (Yamazaki et al. 1991). Mutations in *COL11A2* have been implicated in two distinct human osteochondrodysplasias—an autosomal dominant form (Stickler syndrome) and a recessive form with severe spondyloepiphyseal dysplasia and sensorineural hearing loss (Vikkula et al. 1995). Finally, an association study of a *Bam*HI RFLP polymorphism within *COL11A2* has documented significant ($P = .018$) allelic association with OPLL (Koga et al. 1996) .

Among the 19 molecular variants of *COL11A2* that have been identified thus far, significant associations with OPLL were observed for 4 (table 2), and none of them are obvious disease-causing mutations. The functional impacts of these variants on *COL11A2* expression are currently obscure. A variant in the promoter, at position −182, could potentially affect the transcriptional level of *COL11A2*. The intron 6 variant is located four bases upstream of the splicing junction; therefore, this variant may affect the splicing pattern. The exon 43 variant and the exon 46 variant are silent mutations. Recently, several reports (Wakamatsu et al. 1992; Liu et al. 1997) have suggested the possibility that a silent mutation in an exon has an impact on the splicing pattern; this possibility needs to be examined for the exon 43 variant and the exon 46 variant.

Haplotype analysis is a powerful method for testing the hypothesis that a specific allele mediates predisposition of the disease, especially when causal variants involved in the disease have not been identified. With four distinct diallelic polymorphisms (position −182, intron 6, exon 43, and exon 46), the haplotype was subdivided on the basis of a maximum-likelihood method. Interestingly, very strong significance was found with two haplotypes—namely, H1, with all common alleles at each site, and H4 with all rare alleles at each site (table 3). Conceivably, these results could support the hypothesis that there exists an OPLL-predisposing allele and an OPLL-protective allele, within or near *COL11A2*.

When taken together, these observations of linkage, association, and haplotype analysis show that a genetic locus for OPLL lies close to the HLA region on chromosome 6p in the Japanese population. This study of an OPLL-susceptibility gene is the first step in the identification of this common disease-causing gene. Identification of the disease-susceptibility gene could lead to a better understanding of the molecular pathogenesis of

Table 3

Estimated Haplotype Frequency in OPLL and Non-OPLL Subjects

	Frequency			
Haplotype[a]	OPLL (n = 96 [192 Chromosomes])	Non-OPLL (n = 136 [272 Chromosomes])	χ^2 (df = 1)	P
H1 (++++)	.610	.482	7.38	.0066
H2 (++−+)	.159	.147	.026	.87
H3 (−+++)	.096	.107	.33	.56
H4 (−−−−)	.080	.220	16.86	<.0001
Other	.055	.044	1.65	.20

[a] A plus sign (+) denotes wild-type allele, and a minus sign (−) denotes the mutant allele; the marker order, from left to right, is as follows: position −182, intron 6, exon 43, and exon 46.

this disease and could provide new biological insights into heterotopic bone formation in ligaments.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

ANALYZE, Columbia, ftp://linkage.cpmc.columbia.edu/software/analyze

EMBL-NEW5, http://www.ebi.ac.uk (for U32169)

Whitehead Institute for Biomedical Research / MIT Center for Genome Research, Human Physical Mapping Project, http://www.genome.wi.mit.edu

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